Mechanisms: Chiral

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The separation of racemic compounds into their constituent enantiomers is now routinely performed by high performance liquid chromatography (HPLC) on chiral stationary phases (HPLC-CSPs). HPLC-CSPs are based on molecules of known stereochemical composition immobilized on liquid chromatographic supports. Single enantiomorphs, diastereomers, diastereomeric mixtures and chiral polymers (such as proteins) have been used as the chiral selector.

The first chromatographic separation of an enantiomeric compound on a CSP was reported in 1939 by Henderson and Rule. In this study, racemic camphor was enantioselectively separated on a column containing lactose as the adsorbent. Some of the other significant advances in this field are presented in **Table 1**.

The first commercial HPLC-CSP was developed by Pirkle and introduced by the Regis Chemical Company in 1981. By 1996, the number of commercially available HPLC-CSPs had grown to 110. The rapid increase in the availability of this technology was primarily due to its importance in the discovery, development and regulation of pharmaceutical products.

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The Basis of Chiral Recognition

In order to utilize the wide range of available HPLC-CSPs effectively, it is important to identify the chiral recognition mechanisms operating on these phases. The enantioselective resolutions obtained on CSPs are the result of the formation of temporary diastereomeric complexes between the enantiomeric solute molecules and immobilized chiral selector (solute/CSP). The difference in energy between the resulting diastereomeric *R*-solute/CSP and *S*-solute/CSP complexes determines the magnitude of the observed stereoselectivity, whereas the sum total of the interactions between the solute and CSP, chiral and achiral, determines the observed retention and efficiency.

In order for two enantiomers to be separated chromatographically, there must be a difference in the free energies of binding (ΔG) between the two transient diastereomeric complexes. The energy difference arising from one such interaction would normally be insufficient to permit resolution. However, chromatography is a weighted time-averaged view of many dynamic adsorption–desorption processes. These processes occur throughout the entire length of a column and their sum total can be sufficient to allow an observable difference in the retention times of two enantiomers.

The difference in free energy, $\Delta\Delta G$, needed for adequate chromatographic separation is influenced by the efficiency of the system employed. If the chromatographic system is of high efficiency, so that narrow peaks are observed, relatively small $\Delta\Delta G$ values will afford acceptable analytical-scale enantiomer separations.

For a chiral separation where t_{r1} is the retention time of the first eluting enantiomer, t_{r2} is the retention time of the second eluting enantiomer and t_0 is the retention time of an unretained solute (**Figure 1**). The partitioning of two enantiomerically related analytes between the stationary phase and the mobile phase is defined by the retention factors k_1 and k_2.

$$k_1 = \frac{(t_{r1} - t_0)}{t_0} \qquad [1]$$

Table 1 Some key dates in direct enantioselective separations

1939	Resolution of camphor by column chromatography with lactose as the adsorbent (Henderson and Rule)
1944	Resolution of Troegers base on a lactose column (Prelog and Wieland)
1951	Resolution of amino acid enantiomers by cellulose paper chromatography (Senoh *et al.*)
1966	Resolution of metallocenes on acetylcellulose columns (Falk and Schlogel)
1966	Initial gas chromatographic resolution on a derivatized dipeptide CSP (Gil-Av *et al.*)
1968	Resolution of amino acids on a chiral ion exchange resin (Latt and Rieman)
1971	Resolution of DL-proline by chiral ligand exchange chromatography (Davankov and Rogozhin)
1973	Introduction of microcrystalline triacetylcellulose as a liquid chromatography CSP (Hess and Hegll)
1973	Use of agarose-bonded bovine serum albumin for chiral resolution (Stewart and Doherty)
1976	Resolution of helicines by liquid chromatography on 2-(2,4,5,7-tetranitro-a-fluorenylidone)aminopylpropconic acid (TAPA the first π-acception chiral selector: Gil-Av *et al.*)
1978	Resolution of amino acids on an immobilized chiral crown ether stationary phase (Crom *et al.*)
1979	*N*-formyl-L-valine CSP for HPLC (Hara *et al.*)
1981	(*R*)-*N*-(3,5-dinitrobenzoyl) phenylglycine CSP by Regis Chemical Company first commercially available HPLC-CSP (Pirkle)
1982	Bovine serum albumin-based CSP (Allenmark *et al.*)
1983	α-Acid glycoprotein-based CSP (Hermansson)
1984	Cyclodextrin-based CSP (Armstrong and DeMond)

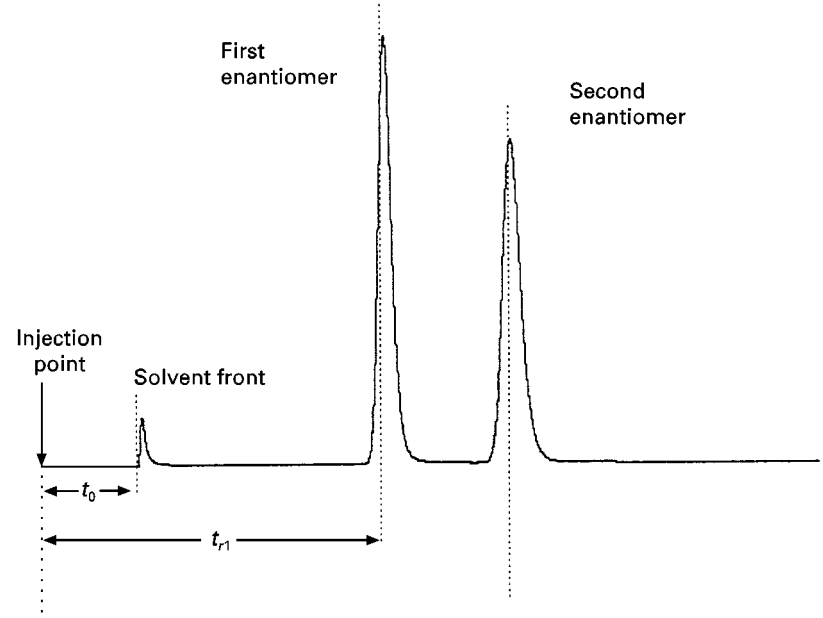

Figure 1 The enantioselective separation of two enantiomers and the measurements required to calculate k_1, k_2 and α.

$$k_2 = \frac{(t_{r2} - t_0)}{t_0} \quad [2]$$

$$\alpha = \frac{k_2}{k_1} \quad [3]$$

$$\Delta G = -RT \ln K \quad [4]$$

where K is the equilibrium constant for the distribution of a solute between the stationary phase and the mobile phase.

$$\Delta\Delta G = \Delta G_2 - \Delta G_1 \quad [5]$$

where ΔG_1 and ΔG_2 are the free energies of binding for the first and second eluting enantiomers, respectively.

$$\Delta\Delta G = -RT(\ln K_2 - \ln K_1) \quad [6]$$

$$\Delta\Delta G = -RT \ln \frac{K_2}{K_1} \quad [7]$$

$$\Delta\Delta G = -RT \ln \frac{k_2}{k_1} \quad [8]$$

$$\Delta\Delta G = -RT \ln \alpha \quad [9]$$

Chiral Recognition Mechanisms

Chiral recognition is a specific aspect of the much broader area of molecular recognition. In chromatographic terms it usually implies the preferential interaction of one solute enantiomer with another enantiomer immobilized on an inert support. The three-dimensional spatial arrangement of the solute requires a complementary three-dimensional structure with which to form a sufficient and necessary number of bonded and nonbonded interactions.

To specify the origin of enantioselective adsorption, one must specify the nature of the various interactions between the species involved. It is also necessary to define a model with which to characterize the requirements of enantioselective recognition. The first model was proposed by Easson and Stedman in 1933. In their mechanism, enantioselective receptor binding was the result of the differential binding of two enantiomers to a common site produced by a three-point contact model between ligand and receptor (**Figure 2**).

In this model, enantiomer 1 was more active than its enantiomorph 2 because 1 was more tightly bound to the receptor (3). The differential binding is a result of the sequence of the substituents, *BCD*, around the chirally substituted carbon atom which forms a triangular face of the tetrahedral bond array. For enantiomer 1, the sequence matches the complementary triad of binding sites on the receptor 3, (*B′C′D′*), leading to a three-point interaction. The enantiomorph 2 has a mirror image sequence, *DCB*, and its interaction with 3 occurs at only two of the three sites on the receptor surface, producing a relatively weaker ligand–receptor interaction.

The three-point interaction model was ignored for 15 years until Ogston resurrected it in order to explain the enzymatic decarboxylation of L-serine to L-glycine. The pivotal step in this conversion was the

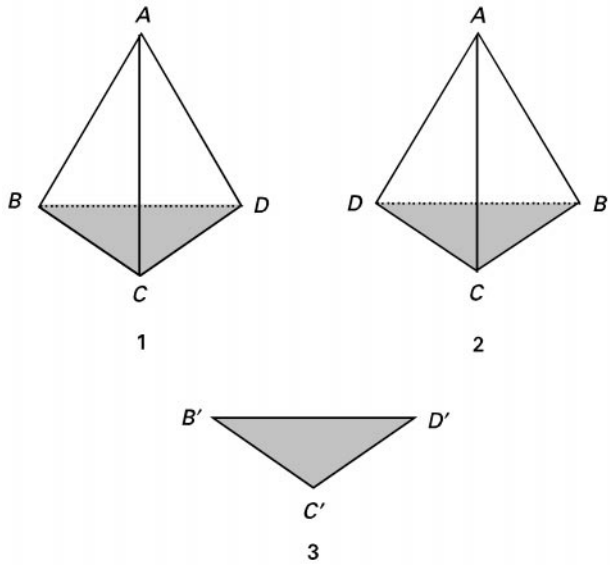

Figure 2 Easson and Stedman's three-point interaction model of chiral discrimination.

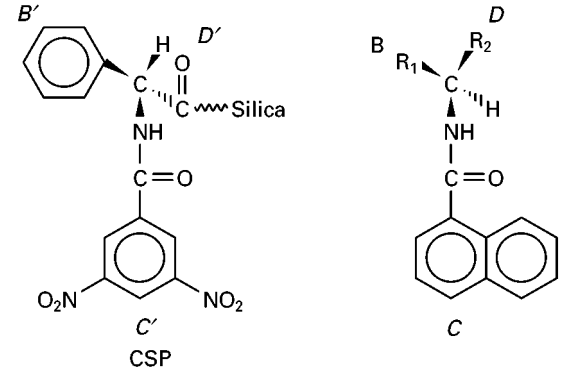

Figure 3 The three-point chiral recognition mechanism, as illustrated by a Pirkle-type CSP.

stereoselective decarboxylation of the prochiral intermediate metabolite aminomalonic acid. In Ogston's model, the carboxylic moieties on the aminomalonic acid become nonequivalent due to the existence of three nonequivalent binding sites on the enzyme, one of which is responsible for the decarboxylation. Ogsten's mechanism was a slight variation of the Easson and Stedman three-point model in that it did not require all three interactions to be attractive.

The first application of the three-point chiral recognition model in chromatography was published by Dalgliesh in 1952. In this work, amino acid enantiomers were resolved by paper chromatography and a three-point mechanism postulated for the interaction between the chiral cellulose stationary phase and the solute enantiomers. Since its introduction into enantioselective chromatography, the three-point interaction model has been the basis for the rational design of a large number of CSPs as well as the basic explanation for the enantioselective separations achieved on them.

Pirkle was the first to exploit this model in the design of synthetic, small molecule CSPs based upon derivatized amino acids. His initial HPLC-CSP was a 3,5-dinitrobenzoyl derivative of phenylglycine (**Figure 3**).

As in the Easson and Stedman model, interactions between the enantiomeric solutes and the CSP take place in the plane defined by $B'C'D'$. However, unlike the enzyme model, all of the attractive interactions are contained along the C' axis, including sites for hydrogen bond donation and acceptance, an amide dipole which can participate in dipole–dipole interactions and the 3,5-dinitrobenozyl moiety which can act as a site for π acid–π base interactions. The phenyl moiety and hydrogen atom on the B' and C' axes are sites of steric interaction.

In the chiral recognition process involving two enantiomers (BCD, DCB) attractive interactions between the amide moieties on the respective C (solute) and C' (CSP) axes create the solute–CSP complexes. The energetic difference between the R-solute–CSP and S-solute–CSP complexes arises from the steric fit of the BD and DB sequences on the enantiomeric solutes into the $B'D'$ sites on the CSP.

It is clear that the three-point interaction model works in a number of situations, especially with small synthetic CSPs. However, when large chiral biopolymers are used as the CSP, this model does not give an accurate description of how the dissymmetry of one molecule is perceived by a second or of how stereochemically equivalent moieties are distinguished from each other. The problem lies in the perception between point asymmetry and molecular asymmetry. In the former approach, a molecule is broken down into its parts, while in the latter it is viewed as the sum of its parts.

In principle, the three-point model is a static picture of a bimolecular process, essentially the lock-and-key model of enzymatic activity. In enzymology, the lock-and-key model has been superseded by the understanding that the pivotal step in enzymatic conversions involves mutually induced conformational adjustments of the substrate and enzyme – an induced molecular fit. When enantiomeric substrates are involved, the differences in enzymatic activity can be related to the energetic differences involved in the formation of the optimum substrate–enzyme complexes and the related transition state energies.

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In analogy with the induced conformational fit utilized in enzyme kinetics, a conformationally driven chiral recognition mechanism has been described for the separation of α-alkyl arylacetic acids on an amylose *tris*(3,5-dimethylphenylcarbamate) CSP (AD-CSP). The chromatographic retentions and enantioselective resolutions of 28 chiral α-alkyl arylacetic acids were related to their respective structures through the construction of quantitative structure–enantioselective retention relationships (QSERR). The QSERR data were combined with molecular modelling studies and the results indicate that the enantioselective discrimination on the AD-CSP proceeds via a three-step process. These steps are:

- Step 1: Distribution of the solute to the stationary phase through hydrogen-bonding interactions between the acid moiety on the solute and amine moieties on the CSP.
- Step 2: Conformational adjustments of the solutes and insertion of the aromatic portion of the solute into a ravine on the CSP.
- Step 3: Stabiliztion of the solute–CSP complex through electrostatic and hydrogen-bonding interactions within the ravine.

This process can be illustrated using the enantioselective separation of *R*- and *S*-benoxaprofen on the AD-CSP. The optimal interaction between *S*-benoxaprofen and the CSP is illustrated in **Figure 4**.

Both *R*- and *S*-benoxaprofen form identical hydrogen-bonding interactions – and presumably the same hydrophobic interactions as well – with the AD-CSP. The energetic differences between the diastereomeric *R*-benoxaprofen–CSP and *S*-benoxaprofen–CSP complexes arise from the internal energies of the two enantiomer conformations which are required to achieve the optimum interactions. The bonding conformation of *R*-benoxaprofen has been calculated to be approximately 250 cal mol^{-1} higher in energy than that of *S*-benoxaprofen. The theoretical enantioselectivity arising from this energy difference was estimated using eqn [9] $\{\Delta\Delta G = -RT \ln \alpha\}$, and the calculated α (1.52) was consistent with the observed α (1.82).

The determining factor in these processes is the molecular chirality of the biopolymer. Enzymes and amylose are large chiral biopolymers with distinct three-dimensional structures. While it is possible to assign specific electrostatic or hydrogen-bonding sites within these molecules, most interactions take place within cavities or ravines. Thus, a more accurate description of the chiral recognition process would be to replace the three-point interaction model with one based on molecular chiralities.

A general chiral recognition process based on this strategy is presented in **Figure 5**. This process involves the initial formation of the complex, followed by conformational adjustment of the two elements, activation of the complex through additional binding interactions and expression of the molecular chiralities of the two elements in the complex. This process describes enantioselective discrimination by all

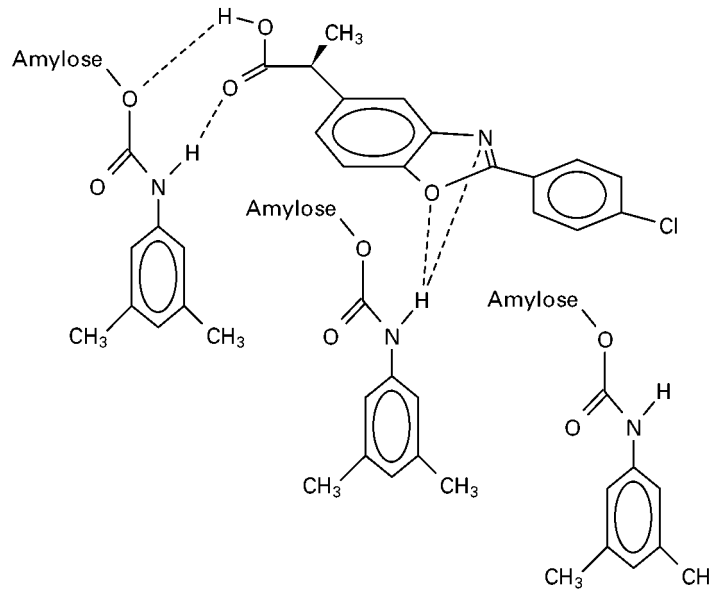

Figure 4 Representative interactions between *S*-benoxaprofen and the AD-CSP, representing a conformationally driven chiral recognition mechanism.

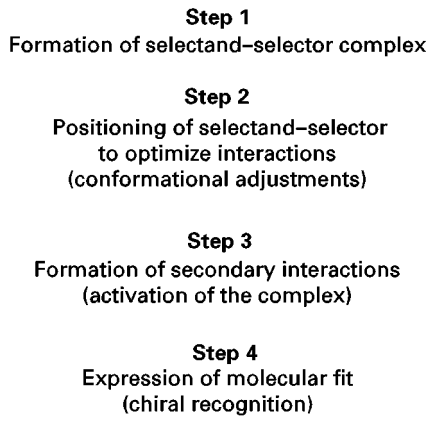

Figure 5 A molecular chiral recognition process.

classes of chiral selectors from biopolymers to derivatized amino acids.

Using Molecular Chiral Recognition to Select a HPLC-CSP

The chiral recognition mechanism presented in Figure 5 can be broken down into its separate parts if one remembers that these parts are interdependent and cannot exist apart from one another. The advantage of considering the steps independently is that it allows for the development of a system for the classification of HPLC-CSPs. If one considers that the key chromatographic step in the chiral recognition process is the formation of the diastereomeric solute–CSP complex, the current HPLC–CSPs can be broken down into five basic types on the basis of the solute–CSP bonding interactions. Using these classes and the molecular structure of the solute, one or more HPLC-CSPs can be selected for the required enantioselective separations. The resulting classes of CSPs are:

- Type I: when the solute–CSP complexes are formed by attractive interactions, such as hydrogen bonding, π–π, dipole stacking, etc., between the solute and CSP. The Pirkle type of CSPs are included in this category
- Type II: when the primary mechanism for the formation of the solute–CSP complex is through attractive interactions, but inclusion complexes also play an important role. The cellulosic and amylosic CSPs are included in this category
- Type III: when the primary mechanism for the formation of the solute–CSP complex is through the formation of inclusion complexes, wherein the sample enters a chiral cavity within the CSP. The cyclodextrin CSPs are included in this category
- Type IV: when the solute is part of diastereomeric metal complex–chiral ligand–exchange chromatography
- Type V: when the CSP is a protein and the solute–CSP complexes are based on combinations of hydrophobic and polar interactions. CSPs based on immobilized α-acid glycoprotein, bovine and human serum albumin and enzymes such as chymotrypsin are included in this category

Conclusion

Research into chiral recognition on CSPs has expanded the original three-point interaction model into a model of molecular chiral recognition.

See Colour Plates 24, 25.

See also: **III/Chiral Separations:** Cellulose and Cellulose Derived Phases; Cyclodextrins and Other Inclusion Complexation Approaches; Ligand Exchange; Liquid Chromatography; Protein Stationary Phases; Synthetic Multiple Interaction ('Pirkle') Stationary Phases.

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Further Reading

Booth TD and Wainer IW (1996) Investigation of the enantioselective separations of α-alkyl arylcarboxylic acids on an amylose *tris*(3,5-dimethylphenylcarbamate) chiral stationary phase using quantitative structure-enantioselective retention relationships (QSERR): identification of a conformationally driven chiral recognition mechanism. *Journal of Chromatography A* 737: 157–169.

Francotte E (1994) Contribution of preparative chromatographic resolution to the investigation of chiral phenomena. *Journal of Chromatography A* 666: 565–601.

Hara S, Nakagawa T and Terabe S (eds) (1994) International symposium on molecular chirality, Kyoto, Japan, 24–27 May 1994. *Journal of Chromatography A* 694.

Jinno K (ed.) (1997) *Chromatographic Separations based on Molecular Recognition*. New York: Wiley-VCH.

Pirkle WH, Pochapsky TC, Burke JA III and Deming KC (1988) Systematic studies of chiral recognition mechanisms. In: Stevenson D and Wilson ID (eds) *Chiral Separations*, pp. 23–36. London: Plenum Press.

Schreier P, Bernreuther A and Huffer M (1995) *Analysis of Chiral Organic Molecules*. Berlin: Walter de Gruyter.

Wainer IW (ed.) (1993) *Drug Stereochemistry*. New York: Marcel Dekker.